

**SECOND SUPPLEMENTAL
DECLARATION**

EXHIBIT C

**ADDITIONAL
DISCLOSURES**

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DISCLOSURES
APPLICATION SERIAL NO. 10/179,589

PAGE 9, LINES 14-16

Morphogenesis or morphogenetics is the origin and evolution of morphological characters and is the growth and differentiation of cells and tissues during development.

PAGE 17, LINE 1 – PAGE 20, LINE 8

EXAMPLE 10

MSX-1 and MSX-2 are the homeobox genes that control the generation and growth of a tooth. A sample of skin tissue is removed from the patient and the MSX-1 and MSX-2 homeobox gene(s) are removed from skin tissue cells. The genes are stored in an appropriate nutrient culture medium.

BMP-2 and BMP-4 growth factors are obtained by recombinant or natural extraction from bone.

Living stem cells are harvested from the bone marrow, the blood of the patient, or from cell culture techniques. The stem cells are placed in a nutrient culture medium at 98.6 degrees. The temperature of the culture medium can be varied as desired but ordinarily is between 40 and 102 degrees F.

MSX-1 and MSX-2 transcription factors are obtained which will initiate the expression of the MSX-1 and MSX-2 homeobox genes.

The MSX-1 and MSX-2 transcription factors, BMP-2 and MBP-4 bone morphogenic proteins, and MSX-1 and MSX-2 genes are added to the nutrient culture medium along with the living stem cells.

EXAMPLE 11

Example 10 is repeated except that the transcription factors bind to a receptor complex in the stem cell nucleus.

EXAMPLE 12

Example 10 is repeated except that the MSX-1 and MSX-2 transcription factors are not utilized. The transcription of the MSX-1 and MSX-2 homeobox genes is activated by applying an electric spark to the nutrient culture medium.

EXAMPLE 13

Example 10 is repeated except that the stem cells are starved and the transcription of the MSX-1 and MSX-2 homeobox genes is activated by applying an electric spark to the nutrient culture medium.

EXAMPLE 14

WT-1 and PAX genes are obtained from a sample of skin tissue removed from the patient. The genes are stored in an appropriate nutrient culture medium. PAX genes produce PAX-2 and other transcription factors.

BMP-7 and other kidney related BMP growth factors are obtained by recombinant or natural extraction from bone.

Living stem cells are harvested from the bone marrow, the blood of the patient, or from cell culture techniques. The stem cells are placed in a nutrient culture medium at 98.6 degrees. The temperature of the culture medium can be varied as desired but ordinarily is between 40 to 102 degrees F.

The WT-1 and PAX genes, and BMP-7 and other kidney BMPS are added to the nutrient culture medium along with the living stem cells.

A primitive kidney germ is produced. The kidney germ is transplanted in the patient's body near a large artery. As the kidney grows, its blood supply will be derived from the artery.

EXAMPLE 15

The Aniridia gene is obtained from a sample of skin tissue removed from the patient. The gene(s) is stored in an appropriate nutrient culture medium.

Aniridia transcription factor (activates expression of the Aniridia gene) and growth factors (function to help stem cells differentiate during morphogenesis to form an eye) are obtained.

Living stem cells are harvested from the bone marrow, the blood of the patient, or from cell culture techniques. The stem cells are placed in a nutrient culture medium at 98.6 degrees. The temperature of the culture medium can be varied as desired but ordinarily is between 40 to 102 degrees F.

The Aniridia transcription factor and growth factor and the Aniridia gene are added to the nutrient culture medium along with the living stem cells.

A primitive eye germ is produced. The eye germ is transplanted in the patient's body near the optic nerve. As the eye grows, its blood supply will be derived from nearby arteries.

EXAMPLE 16

The Aniridia gene is obtained from a sample of skin tissue removed from the patient. The gene(s) is stored in an appropriate nutrient culture medium.

Aniridia transcription factor (activates expression of the Aniridia gene) and growth factors (function to help stem cells differentiate during morphogenesis to form an eye) are obtained and added to the nutrient culture medium.

An eye germ develops. A branch of the nearby maxillary artery is translocated to a position adjacent the eye germ to promote the development of the eye germ. The eye germ matures into an eye which receives its blood supply from the maxillary artery.

The term "cell nutrient culture" as used herein can include any or any combination of the following: the extracellular matrix; conventional cell culture nutrients; and/or, a cell nutrient such as a vitamin. As such, the cell nutrient culture can be two-dimensional, three-dimensional, or simply a nutrient, and is useful in promoting the processes of cellular dedifferentiation, redifferentiation, differentiation, growth, and development.

PAGE 21, LINES 23– 24

An organ, as used herein, consists of two or more kinds of tissues joined into one structure that has a certain task.

PAGE 27, LINES 1-3

In the example above, if germinal cells (and in some cases, stem cells) are utilized a direct differentiation and morphogenesis into an organ can occur *in vivo*, *ex vivo*, or *in vitro*.

PAGE 28, LINES 12-16

Avascular necrosis can be corrected with the insertion of a gene(s) and/or growth factor or other genetic material in the body. For example, avascular necrosis is diagnosed near a joint space. VEGF or BMP genes, or VEGF or BMP growth factors produced by VEGF or BMP genes, respectively, or any other desired genetic based material can be inserted to regrow blood vessels and/or bone.

PAGE 32, LINE 20 – PAGE 39, LINE 19

EXAMPLE 17

A 36-year old Caucasian male experiences pain in his left leg. A medical examination reveals a damaged one-inch long section of a large artery in his left leg. The examination also reveals that this damaged section of the artery is nearly completely clogged with plaque and that the wall of the artery is weakened. The weakening in the arterial wall makes attempting to clean out the artery risky and also makes it risky to attempt to insert a stent in the artery.

Recombinant cDNA encoded to combine with a cell ribosome to produce the human growth factor VEGF is assembled into a eukaryotic expression plasmid. The recombinant

cDNA is from cDNA libraries prepared from HL60 leukemia cells and is known to cause the growth of arteries. The plasmid is maintained at a room temperature of 76 degrees F.

The clones are placed in 1.0 milliliters of a normal saline carrier solution at a room temperature of 76 degrees F. to produce a genetic carrier solution. The genetic carrier solution contains about 250 ug of the cDNA clones. A nutrient culture can, if desired, be utilized in conjunction with or in place of the saline carrier. Each clone is identical. If desired, only a single clone can be inserted in the normal saline carrier solution. The saline carrier solution comprises 0.09% by weight sodium chloride in water. A saline carrier solution is selected because it will not harm the DNA clone.

Two sites are selected for injection of the genetic carrier solution. While the selection of sites can vary as desired, the sites are selected at the lower end (the end nearest the left foot of the patient) of the damaged section of the artery so that the new arterial section grown, can, if necessary, be used to take the place of the damaged section of the artery in the event the damaged section is removed.

The first site is on the exterior wall of the artery on one side of the lower end of the damaged section of the artery. A containment system is placed at the first site.

The second site is inside the wall of the artery on the other side of the lower end of the artery.

The genetic carrier solution is heated to a temperature of 98.6 degrees F. 0.25 milliliters of the genetic carrier solution is injected into the containment system at the first site. 0.25 milliliters of the genetic carrier solution is injected at the second site inside the wall of the artery. Care is taken to slowly inject the genetic carrier solution to avoid entry of the solution into the artery such that blood stream will carry away the cDNA in the solution.

After two weeks, an MRI is taken which shows the patient's leg artery. The MRI reveals new growth at the first and second sites.

After four weeks, another MRI is taken which shows the patient's leg artery. The MRI shows that (1) at the first site, a new artery is growing adjacent the patient's original leg artery, and (2) at the second site, a new section of artery is growing integral with the original artery, i.e., at the second site the new section of artery is lengthening the original artery, much like inserting a new section of hose in a garden hose concentric with the longitudinal axis of the garden hose lengthens the garden hose.

After about eight to twelve weeks, another MRI is taken which shows that the new artery growing adjacent the patient's original artery has grown to a length of about one inch and has integrated itself at each of its ends with the original artery such that blood flows through the new section of artery. The MRI also shows that the new artery at the second site has grown to a length of one-half inch.

In any of the examples of the practice of the invention included herein, cell nutrient culture can be included with the gene, the growth factor, the extracellular matrix, or the environmental factors.

In any of the examples of the practice of the invention included herein, the concept of gene redundancy can be applied. For example, the Examples 1 to 14 concerning a tooth list the genes MSX-1 and MSX-2. These genes differ by only two base pairs. Either gene alone may be sufficient. A further example of redundancy occurs in growth factors. Looking at the Examples 10 to 14, BMP4 or BMP2 alone may be sufficient. Redundancy can also be utilized in connection with transcription factors, extracellular matrices, environmental factors, cell nutrient culture, physiological nutrient cultures, vectors, promoters, etc.

One embodiment of the invention inserts genetic material (gene, growth factor, ECM, etc.) into the body to induce the formation of an organ. Similar inducing materials *ex vivo* into or onto a living cell in an appropriate physiological nurturing environment will also induce the growth of an organ. The VCSEL laser allows early detection in a living cell of a morphogenic change indicating that organ formation has been initiated. With properly time transplantation, organ growth completes itself.

During the *ex vivo* application of the invention, a gene and/or growth factor is inserted into a cell or a group of cells; an ECM or environmental factor(s) are placed around and in contact with a cell or group of cells; or, genetic material is inserted into a subunit of a cell to induce organ growth. An example of a subunit of a cell is an enucleated cell or a comparable artificially produced environment. In *in vivo* or *ex vivo* embodiments of the invention to induce the growth of an organ, the genes, growth factors, or other genetic material, as well as the environmental factors or cells utilized, can come from any desired source.

EXAMPLE 18

Genetically produced materials are inserted in the body to cause the body to grow, reproduce, and replace *in vivo* a clogged artery in the heart. This is an example of site-specific gene expression. A plasmid expression vector containing an enhancer/promoter is utilized to aid in the transfer of the gene into muscle cells. The enhancer is utilized to drive the specific expression of the transcriptional activator. After the enhancer drives the expression of the transcriptional activator, the transcriptional activator transactivates the muscle/artery genes. Saline is used as a carrier. Cardiac muscle can take up naked DNA injection intramuscularly.

Injecting plasmid DNA into cardiac (or skeletal) muscle results in expression of the transgene in cardiac myocytes for several weeks or longer.

Readily available off-the-shelf (RAOTS) cDNA clones for recombinant human VEGF₁₆₅, isolated from cDNA libraries prepared from HL60 leukemia cells, are assembled in a RAOTS expression plasmid utilizing 736bp CMV promoter/enhancer to drive VEGF expression. Other RAOTS promoters can be utilized to drive VEGF expression for longer periods of time. Other RAOTS recombinant clones of angiogenic growth factors other than VEGF can be utilized, for example, fibroblast growth factor family, endothelial cell growth factor, etc. Downstream from the VEGF cDNA is an SV40 polyadenylation sequence. These fragments occur in the RAOTS pUC118 vector, which includes an Escherichia coli origin of replication and the Beta lactamase gene for ampicillin resistance.

The RAOTS construct is placed into a RAOTS 3 ml syringe with neutral pH physiologic saline at room temperature (or body temperature of about 37 degrees C). The syringe has a RAOTS 27 gauge needle.

Access to the cardiac muscle is gained by open-heart surgery, endoscopic surgery, direct injection of the needle with incision, or by any other desired means. The cardiac muscle immediately adjacent a clogged artery is slowly injected with the RAOTS construct during a five second time period. Injection is slow to avoid leakage through the external covering of muscle cells. About 0.5 ml to 1.0 ml (milliliter) of fluid is injected containing approximately 500 ug phVEGF₁₆₅ in saline (N=18). The readily available off-the-shelf cDNA clones cause vascular growth which automatically integrates itself with the cardiac muscle. Anatomic evidence of collateral artery formation is observed by the 30th day following injection to the RAOTS construct. One end of the artery integrates itself in the heart wall to receive blood from the heart.

The other end of the artery branches into increasingly smaller blood vessels to distribute blood into the heart muscle. Once the growth of the new artery is completed, the new artery is left in place in the heart wall. Transplantation of the new artery is not required.

Blood flow through the new artery is calculated in a number of ways. For example, Doppler-derived flow can be determined by electromagnetic flowmeters (using, for example, a Doppler Flowmeter sold by Parks Medical Electronic of Aloha, Oregon) both *in vitro* and *in vivo*. Also, RAOTS angiograms or any other readily available commercial devices can be utilized.

VEGF gene expression can be evaluated by readily available off-the-shelf polymerase chain reaction (PCR) techniques.

If controls are desired, the plasmid pGSVLacZ containing a nuclear targeted Beta-galactosidase sequence coupled to the simian virus 40 early promoter can be used. To evaluate efficiency, a promoter-matched reporter plasmid, pCMV Beta (available from Clontech of Palo Alto, California), which encodes Beta-galactosidase under control of CMV promoter/enhancer, can be utilized. Other RAOTS products can be utilized if desired.

EXAMPLE 19

A patient, a forty-year old African-American female in good health, has been missing tooth number 24 for ten years. The space in her mouth in which her number 24 tooth originally resided is empty. All other teeth except tooth number 24 are present in the patient's mouth. The patient desires a new tooth in the empty "number 24" space in her mouth.

A full thickness mucoperiosteal flap surgery is utilized to expose the bone in the number 24 space. A slight tissue reflection into the number 23 tooth and number 25 tooth areas is carried out to insure adequate working conditions.

A Midwest Quietair handpiece (or other off-the-shelf handpiece) utilizing a #701 XXL bur (Dentsply Midwest of Des Plaines, Illinois) (a #700, #557, #558, etc. bur can be utilized if desired) is used to excavate an implant opening or site in the bone. The implant opening is placed midway between the roots of the number 23 and number 25 teeth. The opening ends at a depth which is about fifteen millimeters and which approximates the depth of the apices of the roots of the number 23 and number 25 teeth. Care is taken not to perforate either the buccal or lingual wall of the bone. In addition, care is taken not to perforate or invade the periodontal ligament space of teeth numbers 23 and 25.

An interrupted drilling technique is utilized to avoid overheating the bone when the #701XXL bur is utilized to form the implant opening. During a drilling sequence, the drill is operated in five-second increments; and the handpiece is permitted to stall. Light pressure and a gentle downward stroke are utilized.

PAGE 44, LINES 8-17

EXAMPLE 35

Example 17 is repeated except that the patient is a 55-year old Caucasian male, and the genetic carrier solution is injected into two sites in the coronary artery of the patient. The first site is on the exterior wall on one side of the artery. The second site is inside the wall of the artery on the other side of the artery. A section of the artery is damaged, is partially blocked, and has a weakened wall. The first and second sites are each below the damaged section of the

artery. Similar results are obtained, i.e., a new section of artery grows integral with the original artery, and a new section of artery grows adjacent the original artery. The new section of artery has integrated itself at either end with the original artery so that blood flows through the new section of artery.